Australian Journal of Entomology (2004) **43**, 184–188

Response of *Culicoides* spp. (Diptera: Ceratopogonidae) to light-emitting diodes

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Abstract Light traps with incandescent globes are used in a national monitoring program to detect the presence of *Culicoides* spp. responsible for the transmission of viruses to livestock and native animals. Recent events have suggested that the efficiency of these traps should be reconsidered and possibly improved. Subsequently, the response of eight species of *Culicoides* to light-emitting diodes (LEDs) was determined at two locations in New South Wales. *Culicoides austropalpalis* Lee & Reye, *C. bunrooiensis* Lee & Reye and *C. marksi* Lee & Reye were attracted to blue light. Responses to blue and green light could not be separated for *C. bundyensis* Lee & Reye, *C. dycei* Lee & Reye, *C. nattiensis* Lee & Reye and *C. victoriae* Macfie. *Culicoides brevitarsis* Kieffer was significantly attracted to green light. This species is the major vector of Akabane and bluetongue viruses in Australia. These responses were all significantly greater than the responses to the incandescent lights currently used in the light traps. The response to red light was less than the response to incandescent light for all species. Catches of *C. brevitarsis* were also related to the intensity of the green LEDs. These were more effective than the currently used incandescent globes at intensities between 46% and 142% of the incandescent intensity.

Key words light traps, monitoring, vectors, viruses.

INTRODUCTION

Many species from the genus *Culicoides* (Diptera: Ceratopogonidae) are of medical or veterinary importance with effects on hosts ranging from mild annoyance to the transmission of viruses. Larvae live in a variety of habitats. Adults exhibit crepuscular activity and commonly enter light traps that are used to study their distribution, behaviour, ecology and importance as vectors (Braverman & Phelps 1981; Zimmerman & Turner 1983; Greiner & Rawlins 1987; Bhatnager *et al*. 1994).

The Australian *Culicoides* fauna is extensive and diverse, and, in many cases, distributions are restricted by geography, weather and habitat availability. Several species from the genus are vectors of viruses affecting native animals. As an example, at least eight individual viruses have been isolated from *Culicoides marksi* Lee & Reye (Standfast *et al*. 1984). *Culicoides brevitarsis* Kieffer is the main species responsible for the transmission of bluetongue and Akabane viruses to livestock (Muller *et al*. 1982). Its distribution is chiefly coastal and it is endemic from the Pilbra region in Western Australia, across the Northern Territory (Muller *et al*. 1981) and down the coastal plains of Queensland to the northern/mid-northern coast of New South Wales (NSW) (Bishop *et al*. 1995b, 1996). Light traps with incandescent globes are used to monitor the presence of the *Culicoides* species and to compare their relative abundances (Dyce *et al*. 1971; Murray 1991; Bishop *et al*. 1995b). The traps are lightweight, designed for easy use in isolated locations and are triggered at sunset by photoelectric cells. However, there have been instances at the margins of the distribution of *C. brevitarsis* in NSW where Akabane activity has been detected by the serological-testing of sentinel cattle herds in the apparent absence of *C. brevitarsis* in light traps (PD Kirkland, pers. comm. 2002). It is possible that these traps fail to record low numbers of infective individuals and that this anomaly could be overcome if the efficiency of the traps could be improved.

The light source in traps for mosquitoes has been investigated by determining mosquito responses to the colour and intensity of conventional light sources and light-emitting diodes (LEDs) (Das & Reuben 1978; Browne & Bennet 1981; Ali *et al*. 1990; Burkett *et al*. 1998). There have been no similar studies on *Culicoides* spp. Olfactory and chemical stimuli have also been added to light traps to improve their efficiency for collecting mosquitoes (Takken & Kline 1989; Kemme *et al*. 1993). Similar responses have been reported for some *Culicoides* spp. (Ritchie *et al*. 1994) and we are considering these effects on *C. brevitarsis* separately.

Insects can generally perceive and respond to light in the 350–700 nm range and their relative response can vary considerably over this range (Land 1997). The standard incandescent light sources used in our light traps generally have a maximum output at 700 nm within this range with little or no output below 400 nm. The response of insect eyes to light

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depends on both photon flux density (PFD) and wavelength. Peak wavelength sensitivities often occur where the output of the incandescent light is low. Recent advances in LEDs in the last 5–10 years have produced LEDs that are energy efficient, often producing a greater total photon flux (TPF) than incandescent globes in the 400–700 nm range for the same power input making them suitable for battery operation. LEDs also have the added advantage that they can provide closely defined outputs across narrow or wide spectral ranges, giving much higher TPF than incandescent globes over certain spectral ranges.

The aim of our study was to determine the response of a range of Australian species of *Culicoides* capable of transmitting viruses to livestock and native animals to different colours in the visible spectrum by the use of LEDs. Improved trapping efficiency, particularly for *C. brevitarsis,* was a major objective.

MATERIALS AND METHODS

Experiments were carried out at Tocal (32°38′S, 151°35′E) and Denman (32°20′S, 150°11′E) in 2002 and 2003 in the Hunter Valley, NSW.

The light source (incandescent globe) in standard light traps was replaced in treatment traps with a range of light-emitting diode (LEDs) treatments and compared to the incandescent lights (see Table 1 for specifications). The traps were powered by three 1.5 V alkaline 'D' cells, which were replaced after two nights of operation. All experiments were carried out when the effects of rainfall, wind speed and moon phase on trap catches were minimal.

The incandescent globes were placed initially in a 110 mm spherical chamber lined with high reflectance white paint. The quantum output was measured at 20°C with a LI-COR Model LI-250 Light Meter with a LI-COR quantum sensor (approximately linear over 400–700 nm) through a port the same size as the active area of the sensor (LI-COR Biosciences, Lincoln, USA). The voltage supplied to the globes was 3.9 V to take into account the 0.6 V drop from the 4.5 V (total batteries) caused in the traps by the switching bipolar silicon transistor. The PFD of the trap with the incandescent globe installed was 0.32 µmol $m^{-2}s^{-1}$ measured with the light meter at 120 mm from the light source in a horizontal plane.

The LEDs (three for blue, green, white and red; and five for yellow) were placed in the same chamber and the current adjusted until the quantum output was the same as the incandescent light source as measured by the quantum sensor. Readings are presented in Table 1. The LEDs other than yellow were mounted in polycarbonate plastic diffusers (120° apart) to ensure more even distribution of light. The yellow LEDs were mounted facing directly outwards on the same plastic caps at 72° apart due to their lower quantum output per current input compared to other LEDs used, possible absorption by the diffusers and restrictions on the power available from the batteries. Current to the LEDs in the traps was controlled by a regulated constant current source.

Trial 1

This trial was conducted at Tocal in March and April 2002 using six light-frequency treatments: blue, green, white, yellow and red LEDs and the standard incandescent globes. The traps were hung from 2 m 'L' shaped frames and placed at 20 m intervals 3 m from one side of the common boundary of two adjacent approximately 30 ha paddocks containing cattle. Four experiments were conducted 3–7 days apart. Yellow was not included in the first two experiments and replaced red in the next two experiments. The five treatments were arranged in five randomised blocks. The positioning of replicates remained constant, while treatments were re-randomised for each experiment. Collections were made into bottles containing 70% ethanol over two nights. *Culicoides brevitarsis* was identified from its wing pattern under × 10 magnification and its total numbers counted and recorded.

Trial 2

Four experiments were conducted, each 2 d apart at Denman in early February 2003. Denman was chosen because it frequently has the greatest diversity of species at sites monitored in coastal NSW (AL Bishop, unpubl. data 2003). It is also marginal for *C. brevitarsis* in most years. Five light-frequency treatments (blue, green, yellow, red and incandescent) were used in this trial. White was omitted because it crossed the ranges of each of the other treatments. The treatments were arranged in five randomised blocks that were re-randomised at the start of each experiment. Traps were hung on 2 m high 'L' shaped frames placed at 12 m intervals on two sides of an approximately 10 ha paddock containing cattle. Collections were made over one night, the samples sorted and numbers of *C. brevitarsis, C. austropalpalis* Lee & Reye, *C. bundyensis* Lee & Reye, *C. bunrooensis* Lee & Reye, *C. dycei* Lee & Reye, *C. marksi, C. nattaiensis* Lee & Reye, and *C. victoriae* Macfie identified from their wing patterns and counted.

Trial 3

Four experiments were conducted at Tocal, 2 d apart in late-February 2003. Green LEDs at four intensities relative to the intensity of incandescent globes were compared with the incandescent light against *C. brevitasis*. The intensities were varied by adjusting the current to the LEDs. The five treatments were arranged in five randomised blocks in a 36 ha paddock containing cattle. The treatments were re-randomised for each experiment. Collections were made over one night and *C. brevitarsis* numbers determined and counted as before.

Statistical methods

The influence of light frequency or intensity on counts of *Culicoides* spp. was modelled using a mixed linear regression approach (Searle 1971), which allowed the separation of variance components into fixed and random effects. To reduce heterogeneity of variances, insect counts were $\log_e$ transformed for Trial 1, Trial 3 and for *C. austropalpalis* in Trial 2.

The square-root transformation was used for counts of all other species in Trial 2 due to their low numbers.

For each trial, analysis of the transformed counts was conducted using the REML directive in Genstat 5.4.1, Release 3. Treatment effects were examined for significance using Wald tests, while treatment means were compared using the least significant difference (LSD) technique at the 5% level and then back-transformed to the original units. The model is given by

y = treatment + experiment + *block* + *block.experiment* + *experiment.treatment* + *block.plot* + *error*

where y = transformed count and the italicised terms are included in the model as random effects.

In Trial 2, where the recorded counts for *C. brevitarsis* and *C. nattiensis* were very low, the counts for the four experiments were pooled for each block and an Analysis of Variance performed.

RESULTS

Trial 1

The treatment effect was highly significant [Wald Statistic (WS) = 178.1, 5 d.f., $P < 0.001$]. Catches of *C. brevitarsis* were highest with green LEDs, lowest with red LEDs and with significant differences between each single-band treatment (Table 2). White was similar to the blue and green treatments but included all wavelengths with peak emissions in the blue and yellow ranges (Table 1).

Trial 2

Significant treatment effects were recorded for each of the eight species at Denman (Table 2). Catches of *C. brevitarsis* (Variance Ratio = 7.62, 3,12 d.f., $P < 0.01$) were again highest with the green LEDs. Numbers of *C. brevitarsis* were low at Denman and were caught in the green treatment on each sampling occasion. They were first caught in the incandescent treatment in the third experiment. *C. austropalpalis* (WS = 375.2, 4 d.f., $P < 0.001$), *C. bunrooiensis* (WS = 38.7, 4 d.f., $P < 0.001$) and *C. marksi* (WS = 247.9, 4 d.f., $P < 0.001$) each exhibited highest responses to blue LEDs, but green LEDs were also more effective than the incandescent light. Significantly higher responses to the blue and green LEDs relative to the incandescent could not be separated for *C. bundyensis* (WS = 40.9, 4 d.f., $P < 0.001$), *C. dycei* (WS = 16.3, 4 d.f., $P < 0.01$), *C. nattiensis* (Variance Ratio = 4.86, 4,16 d.f., $P < 0.01$) and *C. victoriae* (WS = 44.8,4 d.f., $P < 0.01$).

Trial 3

The overall treatment effect of different intensities of green LEDs was significant (WS = 118.5, 4 d.f., $P < 0.001$). Catches increased with intensity, but were not significantly different at the two highest intensities (Table 3). Significantly more *C. brevitarsis* were caught at all intensities tested than in the incandescent traps. These were between 46% and 142% of the incandescent intensity.

DISCUSSION

Light trapping of *C. brevitarsis* was more efficient when incandescent globes were replaced with green LEDs. Attraction was also more effective as the intensity of the green light was increased, with catches at four intensities significantly greater than those with the incandescent light. An upper threshold of intensity suggested by the two highest intensities requires confirmation. Higher PFDs are possible utilising green LEDs than with the incandescent light, given the same power limitations (see Table 1). While trapping of *C. brevitarsis* was the major aim, trapping of seven other species would also be improved with the green LEDs. Specific trapping of some of these species could be maximised with blue LEDs.

Most predictions of the activity and spread of *C. brevitarsis* in NSW are based on population monitoring with light traps and are more dependent on the species occurrence than its density (Bishop *et al*. 1995a, 2000). Larger catches in endemic or established areas where the occurrence of *C. brevitarsis* is not in question might therefore be of little value and the extra time taken to count increased numbers might be unnecessary. Greatest benefit for monitoring use would be derived in areas

Table 1 Specifications and characteristics of light sources tested for attracting species from the genus *Culicoides* in Australia

Source	Nominal colour	Peak emission (400–700 nm)	Light sources per trap	Rated maximum (mA actual per trap)	Total actual current† (mA per trap)	Material	Catalogue number
Incandescent	White	700	1	150 (at 3.5 V)	149 (at 3.9 V)	Tungsten wire	VCH International G191 539752 3.5 V, 0.15 A
LED 'white'	White	460 (main), 570 (secondary)	3	30	69.8	GalnN + fluorescent dye	Z 3981‡
LED 'blue'	Blue	475	3	30	33.4	GalnN	Z 3905‡
LED 'green'	Green	520	3	30	20.6	GalnN	Z 4013‡
LED 'amber'	Yellow	595	5	50	156.0	GaAsP:N	Z 4033‡
LED 'sunset red'	Red	640	3	50	43.0	AlGalnP	Z 4031‡

†To give the same Total Flux Density as the incandescent source. ‡Dick Smith Electronics, Regents Park, NSW, Australia. LED, light-emitting diodes.

Table 2 Predicted (back-transformed) means of *Culicoides* species in response to coloured light-emitting diodes (LEDs) arranged in spectral order in Trial 1 (**) and Trial 2 (*) in the Hunter Valley in 2002 and 2003, respectively, and in relation to the standard incandescent globes. Means in columns with the same letter are not significantly different ($P < 0.05$)

Treatment	Species								
	*C. brevitarsis***	*C. brevitarsis**	*C. austropalpalis**	*C. bundyensis**	*C. bunrooiensis**	*C. dycei**	*C. marksi**	*C. nattiensis**	*C. victoriae**
Red LED	17.0 e	0	5.1 d	0.1 b	0.5 b	2.2 c	1.4 d	0.3 b	0.3 d
Yellow LED	53.5 d	0.7 b	22.4 c	1.0 b	0.4 b	4.1 bc	2.5 cd	0.5 b	3.2 bc
Green LED	279.5 a	4.7 a	52.4 b	5.4 a	0.8 b	8.9 ab	6.4 b	3.1 a	10.6 a
Blue LED	173.5 b	1.3 b	119.0 a	3.9 a	2.2 a	12.7 a	22.2 a	4.9 a	6.3 ab
Incandescent	104.8 c	0.5 b	25.1 c	0.7 b	0.7 b	4.9 bc	3.3 c	0.5 b	1.5 cd
White LED	206.2 ab								

Table 3 Predicted (back-transformed) means of *Culicoides brevitarsis* responding to different intensities of green light-emitting diodes (LEDs) in relation to the intensity of standard incandescent globes. Means with the same letter are not significantly different ($P < 0.05$)

LED: Incandescent TFD ratio (%)	Mean
46	152.4 c
96	208.4 b
115	301.1 a
142	326.3 a
100 (Incandescent)	115.1 d

TFD, Total Flux Density

and at times marginal for *C. brevitarsis,* i.e., for first occurrences outside of endemic areas and at sites with low density. For example, Denman is used as part of the National Arbovirus Monitoring Program (Kirkland *et al*. 1995), which defines the distributions of the bluetongue and Akabane viruses and their vectors for overseas trade requirements. A normal monitoring period is two nights per month at this site. No *C. brevitarsis* were recorded by the incandescent trap in the first two experiments at Denman and a negative report was possible for that month. A trap with green LEDs would have generated a positive report at the site at any time for that month and was 9-fold more effective overall. Traps with green LEDs would therefore have a decided advantage for detecting *C. brevitarsis* in marginal areas and at apparently negative sites where virus has been detected serologically.

Further benefits could be derived where larger catches may be required for virus isolation from vectors, for experimental use of vectors with animals, or for detecting vectors at key locations involved in the export of livestock (staging areas and ports). Colours with higher attraction could possibly be combined with other stimuli currently being investigated or used alone in trapping systems designed to control the insects (e.g., with insecticides, electrified grids or large collection chambers), particularly where important livestock (stud or show animals) are in confined areas such as stables and stalls. Conversely, colours at the other end of the spectrum (red) could possibly provide sufficient light to allow work to continue with animals at night without increasing the attraction of *C. brevitarsis* and the chance that animals would be bitten and infected.

Only eight *Culicoides* species were trapped in these experiments. Other *Culicoides* vectors of the Akabane and bluetongue viruses also exist. These are mainly found in Australia's far north but *C. wadai* Kitaoka has now reached the northern coastal plains of NSW (AL Bishop, unpubl. data 2003). Along with the viruses, these species currently occur within the recorded dispersive limits of *C. brevitarsis*, but this may not always be the case. Coastal and estuarine species are often of human importance and improved trapping efficiency may aid in their study and control. Determination of responses to colour in a wider range of *Culicoides* species throughout Australia and overseas could therefore be an important adjunct to the understanding and control of these pest species and this

could easily be carried out with LEDs in currently used light traps. The variations in response to coloured LEDs we observed may eventually give greater understanding of insect vision and species behaviour.

ACKNOWLEDGEMENTS

Part funding for this researsh was received from Biosecurity Australia.

We thank Drs PD Kirkland (Elizabeth Macarthur Agricultural Institute) and A Gilmour (Orange Agricultural Institute) for their helpful comments during the preparation of the manuscript. The support of staff from the CB Alexander Agricultural College at Tocal and the cooperating farmer at Denman was greatly appreciated.

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Accepted for publication 15 October 2003.